

Article

Antioxidative and Antimycotoxigenic Efficacies of *Thunbergia laurifolia* Lindl. for Addressing Aflatoxicosis in Cherry Valley Ducks

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Abstract: This study aimed to assess the effectiveness of aflatoxin B₁ (AFB₁) and *Thunbergia laurifolia* extract (TLE) in the diets of Cherry Valley ducklings. Our investigation covered growth indicators, blood biochemical indices, meat quality, intestinal morphology, immune response, and CP450 enzyme-related gene expression. We conducted the study with 180 seven-day-old Cherry Valley ducks, randomly divided into five dietary treatments. These treatments included a basal diet without AFB₁ (T1 group), TLE, or a commercial binder; the basal diet containing 0.1 mg AFB₁/kg (T2 group), 0.1 mg AFB₁/kg and 100 mg TLE/kg (T3 group), 0.1 mg AFB₁/kg and 200 mg TLE/kg (T4 group), and 0.1 mg AFB₁/kg and 0.5 g/kg of a commercial binder (T5 group), respectively. Ducklings fed with the T2 diet exhibited lower final body weight (BW), average body weight gain (ADG), and poor feed conversion ratio (FCR) during the 42-day trials. However, all ducklings in the T3, T4, and T5 groups showed significant improvements in final BW, ADG, and FCR compared to the T2 group. Increased alanine transaminase (ALT) concentration and increased expression of CYP1A1 and CYP1A2 indicated hepatotoxicity in ducklings fed the T2 diet. In contrast, ducklings fed T3, T4, and T5 diets all showed a decrease in the expression of CYP1A1 and CYP1A2, but only the T4 treatment group showed improvement in ALT concentration. AFB₁ toxicity considerably raised the crypt depth (CD) in both the duodenum and jejunum of the T2 group, while the administration of 200 mg TLE/kg (T4) or a commercial binder (T5) effectively reduced this toxicity. Additionally, the villus width of the jejunum in the T2 treatment group decreased significantly, while all T3, T4, and T5 groups showed improvement in this regard. In summary, *T. laurifolia* extract can detoxify aflatoxicosis, leading to growth reduction and hepatic toxicosis in Cherry Valley ducklings.

Keywords: antimycotoxigenic; aflatoxin B₁; cherry valley ducks; *Thunbergia laurifolia* extract

Key Contribution: This is the first study to completely evaluate the detoxification ability of *Thunbergia laurifolia* on aflatoxin B₁ through growth performance, biochemical indices, carcass traits, meat quality, intestinal morphology, immune response, and CP450 enzyme gene reaction in Cherry Valley Ducks.



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1. Introduction

Aflatoxins are secondary fungal metabolites, or mycotoxins, primarily produced by toxigenic strains of the fungi *Aspergillus flavus* and *Aspergillus parasiticus* [1]. These mycotoxins are classified as carcinogenic furanocoumarins and consist of twenty related polycyclic structures [2]. Aflatoxin B₁ (AFB₁), the most toxic and prevalent aflatoxin, causes oxidative stress, leading to severe hepatotoxicity. It also inhibits growth and reproductive performance in poultry, resulting in significant negative effects on animal health, food

security, and economic trade [3–5]. Aflatoxins pose a particular problem in hot and dry climates that favor mycotoxigenic fungal growth. Therefore, one of the most severely contaminated areas of AFB₁ in the world is Southeast Asia, especially Thailand, which often experiences higher levels of contamination [6]. Previous research indicated that 38.9% of 3206 samples were highly contaminated with aflatoxin, and the prevalence of aflatoxin reached 44.3% in local corn samples [7].

Poultry aflatoxicosis, traced back to the 1960 outbreak of turkey X diseases in the UK, remains a significant threat to the global poultry industry today [8]. Aflatoxin-contaminated feeds, exacerbated by climate change, continue to cause poor growth performance, compromised reproductive ability, liver necrosis, and bile duct hyperplasia in poultry, leading to substantial economic losses [9]. The detrimental impact extends to bone metabolism, resulting in a weakened skeletal structure and decreased meat yield [10]. Among poultry species, ducklings exhibit the highest sensitivity to AFB₁ [11,12] because waterfowls have high levels of unsaturated fatty acids in their body tissues, making them more susceptible to lipid peroxidation induced by AFB₁ [13–15]. For ducklings, the mortality rates reached 100% at 1 mg/kg AFB₁ [10]. Public health concerns arise from aflatoxin residues in poultry products (e.g., eggs and meat), posing risks ranging from mild liver issues to carcinogenesis in consumers [16]. These challenges underscore the urgent need for stringent regulations and effective mitigation strategies to safeguard poultry welfare and human health while preserving the economic viability of the poultry industry.

The physical characteristics of aflatoxins include high heat stability and polarity [17]. Hence, the efficacy of detoxifying AFB₁ via thermal inactivation is relatively limited. On the other hand, because of the high polarity of aflatoxins, binders exhibit high adsorption ability, making binder supplements the main detoxifying strategy of AFBs in current farms and feed mills [18]. However, binders not only remove AFB₁ but also absorb some nutrition compounds (e.g., zinc and vitamin B group) in feed [19]. The long-term addition of high amounts of adsorbents can cause zinc deficiency, leading to poultry being unable to stand [20]. Phytobiotic feed additives with antioxidant functions appear to be a good choice for detoxifying AFB₁ in poultry. When the feed contains high levels of AFB₁, phytobiotic feed additives with antioxidant functions can neutralize the mycotoxin toxicities for poultry. When the content is low, they can have multiple uses (e.g., improving immunity and growth traits) for birds [21,22].

Thunbergia laurifolia (Rang chuet) extract (TLE) is widely used for neutralizing toxicities from various toxins [23,24]. It is also a common antidote for several poisonous agents in Thai traditional medicine [25,26]. In addition, TLE contains phenolic compounds, which are involved in anti-inflammation and antioxidants [27,28]. Several papers have reported that apigenin, one of the flavonoid compounds in TLE [29], has antioxidant [30] and anticancer properties [31]. The main toxicity of AFB₁ is oxidative stress occurrence via reactive oxygen species production [32]. We hypothesized that TLE had the potential to inhibit aflatoxicosis through its antioxidant ability. In addition, there is no available data about the effects of these herbal medicine products on the duck. Therefore, the poultry industry should develop alternative strategies for detoxifying mycotoxins by TLE in ducks. Hence, the objectives of this research are to comprehensively assess the effects of AFB₁ along with TLE as a natural feed additive in duckling diet on the growth performance, serum biochemical parameters, intestine morphology, carcass traits, meat quality, and immunity responses of Cherry Vally ducks.

2. Results

2.1. Phytochemical and Antioxidant Activity of *T. laurifolia* Extracts

The results showed that the total phenol compound was 0.56 mg GAE/g, and the antioxidant activity, as indicated by the values of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), 2,2-azino-bis (3-ethylbenzothiazole-6-sulfonic acid) (ABTS), and ferric reducing antioxidant power (FRAP), amounted to 7.26 μ mol Trolox equivalents (TE)/g, 3.70, and 51.26 mM Fe²⁺/g, respectively (Table 1).

Table 1. Total phenolic compounds and antioxidant activity of *T. laurifolia* extract.

	Total Phenol Compound (mg GAE/g)	DPPH (μ mol TE/g)	¹ ABTS (IC ₅₀)	FRAP (mM Fe ²⁺ /g)
<i>T. laurifolia</i> extract	0.56 \pm 0.05	7.26 \pm 0.98	3.70 \pm 0.52	51.26 \pm 1.5

¹ABTS: 2,2-azino-bis (3-ethylbenzothiazole-6-sulfonic acid); DPPH: 2,2-diphenyl-1-picrylhydrazyl radical; FRAP: ferric reducing antioxidant power; Fe²⁺: iron divalent ions; GAE: gallic acid equivalents; IC₅₀: half maximal inhibitory concentration; TE: Trolox equivalents.

2.2. Growth Performance

The average daily gain (ADG), the average daily feed intake (ADFI), and the feed conversion ratio (FCR) are presented in Table 2. The final body weight (BW) and ADG were significantly ($p < 0.05$) reduced by AFB₁ during the growth phase (7 to 42 days). However, feeding *T. laurifolia* extract and commercial mycotoxin binder along with AFB₁ significantly improved ADG during days 7–42. There was no significant difference in ADFI between the groups. The FCR during 7 to 42 days was significantly ($p < 0.05$) higher in the AFB₁-challenged groups. Nevertheless, feeding *T. laurifolia* extract and a commercial mycotoxin binder significantly enhanced the FCR compared to AFB₁-fed birds, and it was comparable to that of ducks in the control group.

Table 2. Effects of *T. laurifolia* extract on growth parameters of aflatoxin B₁-challenged ducklings.

Item	¹ T1	T2	T3	T4	T5	SEM	<i>p</i> -Value
Initial BW, g	88.3	82.8	84.7	82.7	86.0	1.61	0.1290
Final BW, g	2303.0 ^a	1831.4 ^b	2244.9 ^a	2307.3 ^a	2236.6 ^a	44.06	<0.0001
ADG, g	63.3 ^a	50.0 ^b	61.7 ^a	63.6 ^a	61.4 ^a	1.82	<0.0001
ADFI, g	145.8	144.9	144.1	139.9	139.7	1.27	0.0880
FCR	2.3 ^b	2.9 ^a	2.3 ^b	2.2 ^b	2.3 ^b	0.08	0.0001

^{a-b} Means with different superscripts in a column differ significantly ($p < 0.05$). ¹T1: Control, only basal diet without AFB₁, TLE or commercial binder; T2: the basal diet containing 0.1 mg AFB₁/kg; T3: the basal diet containing 0.1 mg AFB₁/kg and 100 mg TLE/kg; T4: the basal diet containing 0.1 mg AFB₁/kg and 200 mg TLE/kg; T5: the basal diet containing 0.1 mg AFB₁/kg and 0.5 g/kg of commercial binder; AFB₁: aflatoxin B₁; TLE: *T. laurifolia* extract; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio.

2.3. Blood Biochemistry

Aflatoxin B₁ exhibited significant toxic effects by significantly increasing ($p < 0.05$) the levels of total cholesterol, triglyceride, aspartate transaminase (AST), and globulin (Table 3) in serum biochemical values. When the AFB₁-contaminated diet was supplemented with 100 and 200 mg/kg of TLE or 0.5 g/kg of commercial binder, lower concentrations of AST were observed in the serum of the ducklings compared to those fed without these detoxifying agents ($p < 0.001$). Additionally, the AST values in ducklings fed the AFB₁-contaminated diet with TLE treatment were significantly reduced compared to those of the commercial binder treatment.

Table 3. Effects of *T. laurifolia* extract on serum biochemical of aflatoxin B₁-challenged ducklings.

Item	¹ T1	T2	T3	T4	T5	SEM	<i>p</i> -Value
Total cholesterol, mg/dL	110.0 ^c	151.0 ^{ab}	161.3 ^a	157.7 ^{ab}	129.3 ^{bc}	9.28	0.0135
Triglyceride, mg/dL	122.0 ^c	183.3 ^{ab}	177.0 ^{ab}	158.0 ^{bc}	218.3 ^a	16.51	0.0232
AST, U/L	27.0 ^d	43.3 ^a	33.0 ^c	29.7 ^{cd}	37.7 ^b	1.41	<0.0001
ALT, U/L	32.7 ^{ab}	62.3 ^a	47.3 ^a	32.3 ^b	44.0 ^a	3.17	0.0001
ALP, U/L	846.3 ^a	834.0 ^a	749.7 ^b	662.0 ^c	833.67 ^a	18.05	0.1922

Table 3. Cont.

Item	¹ T1	T2	T3	T4	T5	SEM	p-Value
Total protein, mg/dL	2.73 ^c	2.87 ^{bc}	3.03 ^{abc}	3.30 ^a	3.23 ^{ab}	0.12	0.0431
Albumin, mg/dL	1.37	1.37	1.27	1.43	1.43	0.06	0.2742
Globulin, mg/dL	1.37 ^b	1.67 ^a	1.60 ^{ab}	1.86 ^a	1.80 ^a	0.08	0.0118

^{a–d} Means with different superscripts in a column differ significantly ($p < 0.05$). ¹T1: Control, only basal diet without AFB₁, TLE or commercial binder; T2: the basal diet containing 0.1 mg AFB₁/kg; T3: the basal diet containing 0.1 mg AFB₁/kg and 100 mg TLE/kg; T4: the basal diet containing 0.1 mg AFB₁/kg and 200 mg TLE/kg; T5: the basal diet containing 0.1 mg AFB₁/kg and 0.5 g/kg of commercial binder; AFB₁: aflatoxin B₁; TLE: *T. laurifolia* extract; AST: aspartate transaminase; ALT: alanine transaminase; ALP: alkaline phosphatase.

2.4. Intestine Morphology

Aflatoxin B₁ had significantly unequal effects on the different parts of the examined intestine morphology ($p < 0.01$, Table 4). In general, the villus height (VH) of the duodenum, jejunum, and ileum in ducklings fed the diet containing 0.1 mg/kg AFB₁ was higher than those of ducklings fed the control diet. However, all detoxifying treatments did not decrease the VH but rather increased the values. Notably, AFB₁ increased crypt depth (CD) in the duodenum and jejunum ($p < 0.0001$) but reduced CD in the ileum ($p = 0.0011$), while those fed with the AFB₁-contaminated diet supplemented with 200 mg/kg TLE improved these phenomena. The toxicity of AFB₁ yielded contrasting results in the villus width (VW) of the duodenum and jejunum. Compared to the control group, the VW in the duodenum of the AFB₁-contaminated group was higher ($p = 0.0015$), while the VW in the jejunum of the AFB₁-contaminated group was lower ($p = 0.0028$). Additionally, 200 mg/kg TLE ameliorated AFB₁ toxicity in VW of both parts of the intestine. As for villus height per crypt depth ratio (VH:CD), only the ileum was affected by AFB₁ ($p = 0.013$), while the treatments of 100 mg/kg TLE and 0.5 g/kg commercial binder treatments rather increased the ratio.

Table 4. Effects of *T. laurifolia* extract supplementation on intestinal morphology of aflatoxin B₁-challenged ducklings.

Item	¹ T1	T2	T3	T4	T5	SEM	p-Value
Duodenum							
VH	904.36 ^e	974.41 ^d	989.81 ^c	1069.15 ^b	1098.01 ^a	1.32	<0.0001
VW	115.85 ^d	133.42 ^{ab}	136.02 ^a	126.65 ^c	128.02 ^{bc}	1.08	0.0015
CD	214.37 ^b	222.21 ^a	226.21 ^a	178.33 ^c	166.74 ^d	0.96	<0.0001
VH:CD	4.52 ^c	4.62 ^c	4.75 ^c	6.58 ^b	7.31 ^a	0.10	<0.0001
Jejunum							
VH	775.95 ^e	782.95 ^d	799.55 ^c	857.01 ^b	961.79 ^a	1.15	<0.0001
VW	113.55 ^{ab}	103.52 ^c	109.12 ^b	117.88 ^a	117.59 ^a	0.96	0.0028
CD	174.95 ^b	181.74 ^a	180.64 ^a	167.56 ^c	150.76 ^d	0.97	<0.0001
VH:CD	4.56 ^c	4.53 ^c	4.37 ^c	5.78 ^b	6.57 ^a	0.05	<0.0001
Ileum							
VH	539.73 ^e	616.03 ^d	627.63 ^c	700.65 ^a	650.57 ^b	1.76	<0.0001
VW	98.39	93.37	98.77	94.85	91.33	1.67	0.2557
CD	101.35 ^a	86.01 ^b	90.03 ^b	102.74 ^a	84.81 ^b	1.11	0.0011
VH:CD	6.00 ^d	7.33 ^c	7.93 ^{ab}	7.56 ^{bc}	8.18 ^a	0.11	0.0013

^{a–e} Means with different superscripts in a column differ significantly ($p < 0.05$). ¹T1: Control, only basal diet without AFB₁, TLE or commercial binder; T2: the basal diet containing 0.1 mg AFB₁/kg; T3: the basal diet containing 0.1 mg AFB₁/kg and 100 mg TLE/kg; T4: the basal diet containing 0.1 mg AFB₁/kg and 200 mg TLE/kg; T5: the basal diet containing 0.1 mg AFB₁/kg and 0.5 g/kg of commercial binder; AFB₁: aflatoxin B₁; TLE: *T. laurifolia* extract; VH: villus height; VW: villus width; CD: crypt depth; VH:CD: villus height per crypt depth ratio.

Light microscopy micrographs of the intestine of each experimental group were shown in Figure 1. It was observed that the photomicrograph of the jejunum sections of the control group (T1) showed normal histology of intestinal villi with normal pseudostratified epithelium with goblet cells. In contrast, the addition of 0.1 mg/kg AFB₁ had a significant effect on jejunum tissue histopathology. The photomicrograph of the jejunum section of the T2 group (0.1 mg/kg AFB₁) showed mucosal necrosis. Meanwhile, the photomicrograph of the jejunum section of the T3, T4, and T5 groups (AFB₁ with TLE or commercial binder) showed a marked improvement in mucosal necrosis with an increase in villi integrity, especially in T4 (0.1 mg AFB₁/kg and 200 mg TLE/kg) and T5 (0.1 mg AFB₁/kg and 0.5 g/kg of commercial binder). There were similar results in the ileum sections. The T1 group had relatively complete and compact villus tissue. The T2 group had a looser villus structure than the T1 group due to aflatoxicosis in the ileum villus structure. The T4 and T5 groups had the effect of improving AFB₁ toxicity.

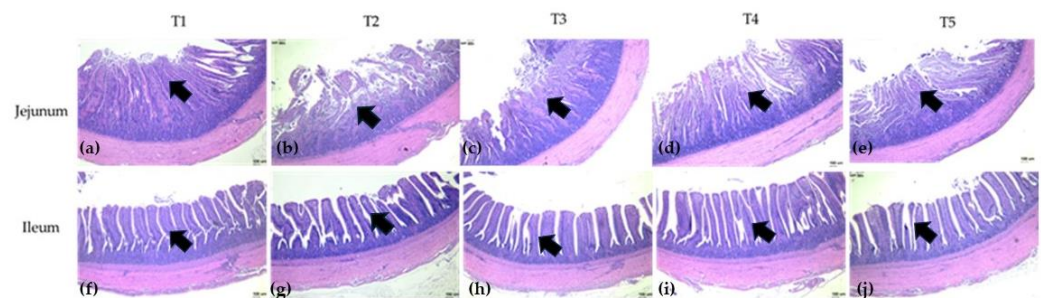


Figure 1. Histological representations of the H&E-stained jejunum and ileum sections of ducks. (a) T1: Control, only basal diet without AFB₁, TLE, or commercial binder, which showed normal histology of intestinal villi with normal pseudostratified epithelium with goblet cells (arrow) in jejunum; (b) T2: the basal diet containing 0.1 mg AFB₁/kg, which AFB₁ showed significant mucosal necrosis and decreased villi integrity in the jejunum (arrow); (c) T3: the basal diet containing 0.1 mg AFB₁/kg and 100 mg TLE/kg, which showed mild mucosal necrosis and loose villi integrity in the jejunum (arrow); (d) T4: the basal diet containing 0.1 mg AFB₁/kg and 200 mg TLE/kg, which showed slight mucosal necrosis and loose villi integrity in the jejunum (arrow); (e) T5: the basal diet containing 0.1 mg AFB₁/kg and 0.5 g/kg of commercial binder, which showed slight mucosal necrosis and loose villi integrity in the jejunum (arrow). (f) T1 showed the complete and compact villus tissue in the ileum (arrow); (g) T2 showed loose villus structure in the ileum (arrow); (h) T3 showed slightly loose villus structure in the ileum (arrow); (i) T4 showed slightly loose villus structure in the ileum (arrow); (j) T5 showed slightly loose villus structure in the ileum (arrow); Magnification was 10× the objective lens. Scale bars represent 100 µm.

2.5. Carcass Trait, Relative Organ Weight, and Meat Quality

The *T. laurifolia* extract and AFB₁ supplementation did not influence the relative weight of carcass (excluding neck and feet), breast meat, bursa of Fabricius, or spleen, but there was a tendency for an increase ($p < 0.1$) in liver and gizzard weight. The relative weight of the bursa of Fabricius, spleen, breast meat, and carcass (excluding the neck and feet) was not affected by the *T. laurifolia* extract or AFB₁ supplementation; however, there was a tendency for the liver and gizzard weight to increase ($p < 0.1$) in duckling fed with T2 and T3 (Table 5). Dietary treatments did not affect the pH test for 45 min and 24 h, thiobarbituric acid reactive substances (TBARS), lightness (L), redness (a), or drip loss (Table 6). However, the inclusion of AFB₁ increased ($p < 0.05$) shear force and breast meat yellowness (b).

Table 5. Effects of *T. laurifolia* extract on carcass trait and relative organ weight of aflatoxin B₁-challenged ducklings.

Item	¹ T1	T2	T3	T4	T5	SEM	<i>p</i> -Value
Carcass traits							
Dressing, %	87.73	86.23	87.30	87.65	85.90	0.57	0.0822
Breast, g	10.81	9.75	10.02	10.48	11.20	0.52	0.2814
Thigh, g	7.49	7.71	7.90	8.22	7.82	0.29	0.4990
Wing, g	12.07	12.07	12.19	12.40	12.56	0.22	0.4555
Relative organ weight, g							
Liver, g	2.24	2.34	2.28	2.04	2.17	0.08	0.0696
Spleen	0.11	0.09	0.09	0.09	0.09	0.01	0.5229
Kidney	0.70	0.74	0.75	0.76	0.72	0.02	0.4222
Bursa of fabricius	0.17	0.18	0.16	0.18	0.17	0.01	0.7154
Heart	0.61	0.65	0.63	0.66	0.65	0.02	0.6874
Gizzard	4.71	4.75	4.79	4.49	5.07	0.14	0.0918

¹T1: Control, only basal diet without AFB₁, TLE or commercial binder; T2: the basal diet containing 0.1 mg AFB₁/kg; T3: the basal diet containing 0.1 mg AFB₁/kg and 100 mg TLE/kg; T4: the basal diet containing 0.1 mg AFB₁/kg and 200 mg TLE/kg; T5: the basal diet containing 0.1 mg AFB₁/kg and 0.5 g/kg of commercial binder; AFB₁: aflatoxin B₁; TLE: *T. laurifolia* extract.

Table 6. Effect of *T. laurifolia* extract on meat quality of aflatoxin B₁-challenged ducklings.

Item	¹ T1	T2	T3	T4	T5	SEM	<i>p</i> -Value
pH value _{45 min}	5.91	6.16	6.21	5.95	5.90	0.12	0.2494
pH value _{24 h}	5.60	5.58	5.66	5.57	5.55	0.06	0.6975
Cook loss, %	30.60	31.81	32.52	33.91	33.95	1.30	0.3258
Drip loss, %	2.83	2.20	2.56	2.42	2.48	0.66	0.9743
Shear force, N	32.20 ^b	34.20 ^b	29.48 ^b	32.37 ^b	45.44 ^a	3.35	0.0374
TBARS, mg MDA/kg	1.59	1.45	1.50	1.55	1.54	0.06	0.5031
Meat color							
L*	40.50	42.20	43.00	38.80	42.15	1.74	0.4822
a*	15.90	16.79	16.28	14.83	16.71	0.64	0.2671
b*	4.03 ^c	7.23 ^a	7.04 ^a	4.36 ^{bc}	6.46 ^{ab}	0.72	0.0258

^{a-c} Means with different superscripts in a column differ significantly ($p < 0.05$). ¹T1: Control, only basal diet without AFB₁, TLE or commercial binder; T2: the basal diet containing 0.1 mg AFB₁/kg; T3: the basal diet containing 0.1 mg AFB₁/kg and 100 mg TLE/kg; T4: the basal diet containing 0.1 mg AFB₁/kg and 200 mg TLE/kg; T5: the basal diet containing 0.1 mg AFB₁/kg and 0.5 g/kg of commercial binder; AFB₁: aflatoxin B₁; TLE: *T. laurifolia* extract; TBARS: thiobarbituric acid reactive substances; MDA: malondialdehyde; L*: lightness; a*: redness; b*: yellowness.

2.6. Expression of Immune Response and Metabolizing Cytochrome P450 Enzyme-Related Genes

T. laurifolia extract mitigated liver pathological damage caused by AFB₁ in ducklings. The mRNA levels of the inflammation-related gene (TNF α) in the liver were significantly upregulated in ducks treated with AFB₁ compared to those in the control and TLE groups (Figure 2). Additionally, the mRNA expression levels of CYP1A1 and CYP1A2 in the liver were increased in the AFB₁ group compared with those of the control group.

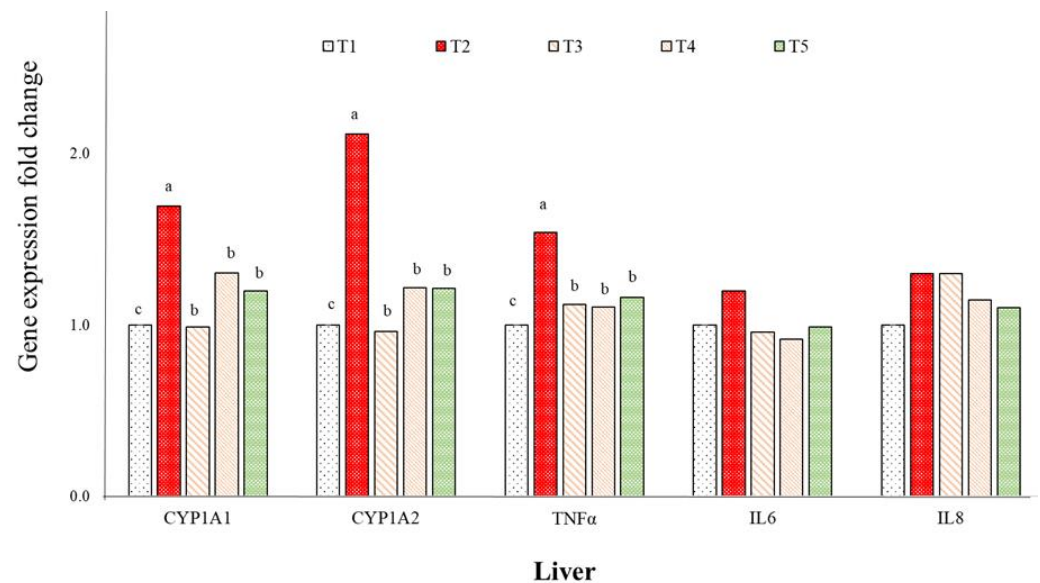


Figure 2. Expressions of immune (tumor necrosis factor α , TNF α ; interleukin 6, IL6; interleukin 8, IL8) and metabolizing Cytochrome P450 enzyme (CYP1A1 = cytochrome P450 1A1; CYP1A2 = cytochrome P450 1A2) related genes in liver of aflatoxin-challenged ducks. Three replicates. ^{a–c} Columns without the same superscripts differ ($p < 0.05$). T1: Control, only basal diet without AFB₁, TLE, or commercial binder; T2: the basal diet containing 0.1 mg AFB₁/kg; T3: the basal diet containing 0.1 mg AFB₁/kg and 100 mg TLE/kg; T4: the basal diet containing 0.1 mg AFB₁/kg and 200 mg of TLE/kg; T5: the basal diet containing 0.1 mg AFB₁/kg and 0.5 g/kg of commercial binder; AFB₁: aflatoxin B₁; TLE: *T. laurifolia* extract.

3. Discussion

3.1. Antioxidative Capacity of *T. laurifolia* Extract

The *T. laurifolia* extract is a traditional Thai herbal medication known for its antioxidative capacity [33]. One of the main active ingredients of TLE is total phenolic compounds. A previous study [34] indicated a positive correlation exists for other antioxidant capacity methods, such as DPPH and FRAP with polyphenols. The present examination not only investigated the antioxidative capacity of TLE by determining the ABTS, DPPH, and FRAP but also tested the active compound phenolic content. The TLE of the current study exhibited lower activities in terms of DPPH, ABTS, and total phenolic compounds compared to another study [35]. While probiotics offer various significant benefits for livestock health, their drawback lies in the variability of composition influenced by factors such as harvesting season and geographical location [36]. This variability may also be one of the reasons why a higher concentration (200 mg/kg TLE) was required to have a noticeable AFB₁ detoxification effect in this trial.

3.2. Aflatoxin B₁ Toxicity on Growth Performance

The regulatory limit for AFB₁ in the EU, FDA, and China is 0.02 mg/kg for ducklings [37–39]. However, this limit level serves as a precautionary measure to prevent the potential harmful accumulation of AFB₁ in the bodies of animals after long-term ingestion (over four weeks). Previous research has indicated that AFB₁ concentration can impair duck production, and significant hepatic lesions can occur at levels as low as 0.5 mg/kg for a short period (lower than four weeks) [40,41]. Taking into account the treatment period (five weeks), experimental efficiency, and various national regulations, we compromised and chose 0.1 mg/kg as the tested content.

It is well established that AFB₁ can interfere with poultry energy metabolism, reducing growth efficiency [36,37]. Among poultry, meat ducks are susceptible to aflatoxins. A diet containing a high concentration of AFB₁ can cause acute death in meat-type ducks, while

prolonged exposure to low levels of AFB₁ can induce chronic toxicity, resulting in growth retardation and reduced production [42]. Previous research has indicated that poultry-fed diets containing aflatoxins as low as 0.3 mg/kg started to show reductions in growth rate, and feed intake and feed efficiency worsened [43]. In the current study, the results indicated that a diet containing 0.1 mg/kg of AFB₁ led to a reduction in ADG and poor FCR in ducklings. Unlike ADG and FCR, the ADFI of ducklings remained unaffected by AFB₁ toxicity, which aligns with the effects of AFB₁ on early young broiler research [44].

3.3. Aflatoxin B₁ Toxicity on Serum Biochemical Parameters

Hepatotoxicity is the primary characteristic of AFB₁ toxicity in numerous animal species [5]. Blood AST, ALT, and alkaline phosphatase (ALP) levels are commonly used as indicators when measuring the effects of aflatoxin on liver toxicity in poultry [45]. Globulin involves several physiological processes, including lipid transportation in birds [15]. Our study revealed that AFB₁ altered serum biochemical parameters, leading to significantly higher levels of total cholesterol, triglycerides, AST, and globulin. However, the levels of ALT and ALP in the AFB₁ group did not show a significant increase compared to the control group in our study. This may be attributed to the AFB₁ concentration in this research not reaching the toxic level required for severe liver damage, which would release high amounts of ALT and ALP. The results of the relative liver weight in our experiment support this. Although the liver weights of the AFB₁ group were heavier than those of the control and other treatment groups, the difference was not statistically significant. Similar results were observed in other experiments. For instance, adding over 0.5 mg/kg of AFB₁ to broiler diets can increase serum ALP, ALT, and AST activities [46]. However, when the dietary AFB₁ concentration was lower than 0.03 mg/kg, only serum AST levels were significantly increased in broilers [5]. The AFB₁-induced increase in serum total cholesterol and triglycerides observed in this study is consistent with previous research findings [47,48]. The liver plays a crucial role in blood fatty acid metabolism [49], while AFB₁ induces liver damage and can lead to abnormal triglyceride metabolism.

3.4. Aflatoxin B₁ Toxicity on Intestine Morphology

Aflatoxin B₁ can alter intestinal morphology, leading to reduced nutrient absorption and subsequent growth retardation [50,51]. However, the effects of AFB₁ toxicities on poultry intestinal morphology are not entirely clear. This lack of clarity may stem from differences in the specific sections of the intestine, tested variables, and exposure time in previous studies [46]. Additionally, the species and age of poultry used in various studies may also play crucial roles in the intestine's response to chronic aflatoxicosis. An earlier study indicated that AFB₁ can induce morphological alterations of the intestinal epithelium by increasing the depth of the crypts, particularly in the small intestine (duodenum and jejunum) [52]. While these findings were consistent with the observations in the duodenum and jejunum, they did not align with those of the ileum in the present study. Furthermore, most research has indicated that AFB₁ decreased VH in the small intestine of broilers. However, contrary to the observations in broilers [46,52], AFB₁ had no effect on VH in laying hens [53]. The results of our meat duckling trial also differed from those of the broiler chicken test. Surprisingly, the VH of the duodenum, jejunum, and ileum were all significantly increased by AFB₁ toxicity. Alterations in both the height and width of villi were also noted in ducks treated with AFB₁. The alterations in the structure of villi were a result of the activation of the apoptotic pathway by AFB₁, which subsequently may be related to the absorption of nutrients. Given the differences in these results of intestinal morphology, in addition to the abovementioned differences in varieties and sampling locations, further testing may be necessary to verify and confirm these findings.

3.5. Aflatoxin B₁ Toxicity on Carcass Traits and Meat Quality

Several interesting results were observed regarding carcass traits and meat quality. In contrast to other reports [5,10], our results did not show significant changes in the relative weights of the liver and other organs. Although there was a slight increase in the AFB₁-contaminated treatment group compared with the control group, this difference did not reach statistical significance. This could be attributed to the tested concentrations of AFB₁ in this study causing mild hepatotoxicity that did not reach the threshold to alter liver weight. In terms of meat quality, it was discovered that the color of the meat in the AFB₁-contaminated group showed a significant increase. To the best of our knowledge, there were no other poultry reports that investigated whether AFB₁ changes the color of poultry meat. However, we found a sheep report [54] indicating that AFB₁ altered the lightness (L value) of the meat but not the yellowness (b value). Although there were slight differences between the results of the former study and ours, these variations may be attributed to differences in animal species. Nonetheless, it is plausible that AFB₁ could indeed cause changes in meat color. We speculated that disruptions in pigment metabolism and inflammatory responses associated with liver damage could also influence the color of the meat, potentially contributing to changes in its yellowness [55].

3.6. Aflatoxin B₁ Toxicity on Immunity and Cytochrome P450 Enzyme-Related Genes

Aflatoxin B₁ induces oxidative damage and apoptosis in hepatocyte cells and is primarily metabolized by cytochrome P450 (CYP450) enzymes [56]. In poultry liver, AFB₁ is bioactivated by enzymes such as CYP1A1, CYP1A2, and other enzymes (e.g., CYP2A6 and CYP3A4). CYP450 enzymes convert AFB₁ into an electrophilic, highly reactive, and unstable metabolite known as aflatoxin-8,9-epoxide (AFBO) [57,58]. This metabolite can interact with cellular macromolecules, binding to guanine residues in DNA, causing genotoxicity, and reacting with proteins to induce cytotoxicity [59]. These interactions result in irreversible DNA damage and can lead to hepatocarcinoma in humans, primates, and ducks [60]. Consistent with previous research in broiler chickens [61], our study observed that AFB₁ exposure led to a significant increase in CYP1A1 and CYP1A2 mRNA expression. Additionally, our findings were consistent with previous studies, which demonstrated that AFB₁ treatment increased the mRNA levels of TNF- α [62,63]. This indicates that AFB₁ toxicity induces the immune response and inflammatory cytokine production in ducklings. However, the mRNA expressions of these enzymes and TNF- α were lower in groups treated with TLE and a commercial binder, suggesting that these feed additives effectively neutralize the hepatotoxic effects of AFB₁.

3.7. Antimycotoxigenic Efficacies of *Thunbergia laurifolia* Lindl.

Aflatoxin B₁ is primarily metabolized through CYP1A1 and CYP1A2, producing a highly reactive intermediate (AFBO), which induces the formation of reactive oxygen species (ROS) within hepatocytes [64]. The accumulation of ROS leads to oxidative stress, characterized by an imbalanced response between the production of reactive species and the ability of cells to detoxify or repair the damage [65]. Reactive oxygen species damage cellular components, including lipids, proteins, and DNA, initiating lipid peroxidation and compromising membrane integrity, ultimately leading to cell damage and death [66]. Oxidative damage and cellular stress induce a series of inflammatory responses in the liver, further aggravating tissue damage. Liver damage impairs critical functions, such as detoxification, protein synthesis, and nutrient metabolism, leading to reduced nutrient absorption and utilization, which contributes to poor growth performance [67].

Therefore, *T. laurifolia* with natural antioxidants may be a promising option to neutralize AFB₁ toxicity. Much research has indicated that *T. laurifolia* possesses antioxidant and anti-inflammatory properties, as well as anticancer activities, due to its ability to increase catalase (CAT) and glutathione peroxidase (GPx) activities, thereby removing ROS [68–70]. Previous research on chickens has shown promising results using 2% *T. laurifolia* leaf [71]. This treatment ameliorated the adverse effects of multiple mycotoxin-contaminated feeds,

improving nutrient digestibility and increasing the activity of glutathione peroxidase. However, it did not lead to a significant change in the growth rate. Our research further investigated the potential of TLE in mitigating the effects of AFB₁ on growth reduction and hepatotoxicity. By utilizing extracts of *T. laurifolia* in our study, we hypothesized that some impurities were eliminated to enhance the concentration of bioactive chemicals, such as total phenolic compounds [72]. Therefore, we only used 100 mg/kg TLE to improve the growth reduction caused by AFB₁, and the treatment of 200 mg/kg TLE had a stronger detoxification ability, as observed in growth performance, serum biochemical traits, intestinal morphology, and meat quality.

Our results suggest that supplementing TLE into duckling diets could be a natural and effective detoxifying agent against AFB₁ contamination. This can lead to improved growth performance, feed efficiency, and overall health in poultry, which is crucial for the poultry industry. Additionally, the study presented that TLE improves meat quality by mitigating the adverse effects of AFB₁. This is critical for ensuring that the meat produced is safe and high quality. Our findings pave the way for further research into the use of TLE for detoxifying various mycotoxins in different animal species.

4. Conclusions

It can be concluded that dietary supplementation of *T. laurifolia* extract in ducklings ameliorated the adverse effects of AFB₁ on growth performance, alleviated liver damage by increasing the drug-metabolizing enzymes (Cytochrome P450), and improved the intestinal health of ducks through participation in their detoxification.

5. Materials and Methods

5.1. Animal and Ethical Approval

A total of 180 seven-day-old Cherry Valley ducks were obtained from the Faculty of Agriculture, Chiang Mai University, Thailand. The ducks were housed in pens with strict biosecurity measures, with each treatment containing 3 replications of 12 birds each. Over the 35-day duration of the experiment, the ducks received water and feed ad libitum (Table 7). All experimental procedures in this study were conducted strictly in accordance with the recommended guidelines and were submitted for ethical approval by the Animal Ethics Committee, Faculty of Agriculture, Chiang Mai University.

Table 7. The formulation and proximate composition of the experimental diet (g/kg).

Items	1–3 Weeks	4–5 Weeks
Ingredient (g/kg feed)		
Corn meal	700.00	575.00
Rice bran	0.00	75.00
Full-fat soybean meal	0.00	25.00
Soybean meal, 44%	205.00	192.50
Meat meal, 50%	25.00	25.00
Limestone	10.00	25.00
Calcium carbonate	0.00	47.40
Monopotassium phosphate, 22%	10.50	17.50
¹ Premix	2.50	2.50
Methionine	0.90	1.50
Toxin binder	1.00	0.50
Salt	0.00	2.00
Multi protein plus, 68%	45.00	11.00
Phytase	0.10	0.10
Total	1000.00	1000.00

Table 7. Cont.

Items	1–3 Weeks	4–5 Weeks
Nutrient composition (% dry matter basis)		
Moisture	12.23	9.78
Ash	6.79	11.91
Crude protein	22.22	18.0
Crude fiber	4.56	3.82
Crude fat	5.15	4.59
Gross energy (Cal/g)	2964.92	3581.65

¹ Vitamin premix (per kg premix): vitamin A 19,000,000 IU, vitamin D3 3,900,000 IU, vitamin E 11,500 IU, vitamin K₃ 4.30 g, vitamin B₁ 5.50 g, vitamin B₂ 10.50 g, vitamin B₆ 4.80 g, vitamin B₁₂ 0.19 g, vitamin C 15.50 g, pantothenic acid 15.10 g, folic acid 2.90 g, nicotinic acid 39.00 g, biotin 0.25 g. 2 Mineral premix (per kg premix): magnesium 105.00 g, potassium 89.00 g, sodium 105.00 g, and feed additive 24.50 g.

5.2. Plant Materials

The mature leaves of *T. laurifolia* Lindl. were collected from Hangdong District, Chiang Mai Province, Thailand. The leaves were cleaned, chopped into pieces, and then oven dried at 60 °C for 24 to 48 h. Subsequently, the dried leaves were powdered using a dry grinder to obtain particles of approximately 0.2 mm in size. The powdered material was stored in a light-resistant container until it was used for the extraction studies.

5.3. Extraction Method and Phenolic Content Measurement

The procedure involved soaking the powdered *T. laurifolia* leaves in boiling distilled water (1:10 *w/v*) for one hour. Subsequently, the mixture was passed through a filter paper (Whatman No. 41) and three layers of gauze. The filtrate obtained was freeze-dried and kept in a desiccator at a temperature of 4 °C. To facilitate future use, the extract was diluted in distilled water to achieve the appropriate concentrations and then stored at a temperature of −20 °C. The Folin–Ciocalteu technique [73] was employed to quantify the total phenolic content. The extract was combined with the Folin–Ciocalteu reagent and a 7.5% (*w/v*) solution of NaCO₃. The calibration standard for gallic acid was established by incubating it for 60 min and using a UV–Vis spectrophotometer (SPECTROstar Nano, BMG LABTECH, Ortenberg, Germany). The extract's total phenolic content was determined in milligrams of gallic acid per gram.

5.4. Antioxidative Assays

The DPPH and ABTS radical scavenging activities were evaluated using modified methods based on Sunanta et al. [74] and Sangta et al. [44], respectively. For the DPPH assay, 25 µL of the extract was mixed with 250 µL of 0.20 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) solution. The mixture was then incubated at room temperature, in the dark, for 30 min, and the absorbance was measured at 517 nm. Regarding the ABTS assay, 200 µL of the extract was mixed with 500 µL of a working solution containing 7.00 mM ABTS [2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)] and 2.45 mM potassium persulfate. The mixture was incubated in the dark at room temperature for 12–16 h, and the absorbance of the samples was measured at 734 nm. The FRAP was determined using the modified Aljadai method [75]. In this method, 10 µL of the extract was mixed with 190 µL of FRAP reagent for 30 min in the dark, and the absorbance was measured at 593 nm using ascorbic acid as a standard reference.

5.5. Treatment Diet Preparation

The powder of AFB₁ standard (purity ≥ 98%) and commercial binder (Mycosorb Advance) were purchased from Sigma (Saint Louis, MO, USA) and American Colloid Company (Lovell, WY, USA), respectively. One milligram of AFB₁ standard was dissolved in 100 mL of 95% ethanol (Merck, Darmstadt, Germany) to obtain 10 mg/kg AFB₁ stock solutions. The prepared solution was then sprayed evenly on the basal feed and mixed to obtain the 0.1 mg/kg AFB₁-contaminated diet [76,77]. The equivalent amount of ethanol

without AFB₁ solution was sprayed evenly on the basal feed to obtain the control diet. The treatment concentration of TLE and the commercial binder were calculated, respectively, added uniformly to the diet, and mixed evenly. Mycotoxins were detected in the basal diet using ELISA kits (R-Biopharm, Darmstadt, Germany). The analysis revealed that the quantities present in the sample were as follows: 0.012 mg/kg AFB₁, 0.0212 mg/kg T-2 toxin, 0.015 mg/kg ochratoxin A, 0.035 mg/kg zearalenone, and 0.015 mg/kg deoxynivalenol, respectively.

5.6. Growth Performance

All ducklings were fed treatment diets for 35 days. The ducks were clinically observed at least twice daily, and mortality was recorded. Furthermore, the ducks were individually weighed on the age of day 7 and day 42. The performance variables measured in this study include BW, ADG, ADFI, and FCR.

5.7. Blood Characteristics

Blood samples were collected at day 42 from each treatment (6 birds) for biochemical analyses. The blood samples were then centrifuged at $3000 \times g$ for 15 min, and the serum was separated to determine liver function parameters such as AST, ALT, ALP, total protein, globulin, and albumin. All blood characteristics were measured using a BioMajesty[®] JCA-BM6010/C kit from DiaSys Diagnostic Systems (Holzheim, Germany) with an automated chemistry analyzer BX-301 (Asia Green, Singapore).

5.8. Relative Organ Weight

Following the bleeding process, all ducks from each treatment were euthanized via cervical dislocation. Subsequently, the liver, kidney, heart, spleen, gizzard, and bursa of Fabricius were removed, and their weights were measured. The organs were weighed, and their weights were represented as relative organ weights:

$$\text{Relative weight} = (\text{Organ weight}) / (\text{Final BW}) \times 100.$$

5.9. Carcass and Meat Quality

After 42 days of testing, each duck was carefully weighed before being exsanguinated and sacrificed via cervical dislocation. The weight of the carcass (excluding the neck and feet), breast meat, liver, gizzard, pancreas, thymus, bursa of Fabricius, spleen, and abdominal fat was extracted and measured after being rinsed with saline solution. Organ size was quantified as a proportion of BW. The pH of the breast meat was determined using a calibrated glass-electrode pH meter (WTW pH 340-A, WTH Measurement Systems Inc., Ft. Myers, FL). The lightness (L*), redness (a*), and yellowness (b*) values of the breast meat were measured using a Minolta CR410 Chromameter from Konica Minolta Sensing Inc., located in Osaka, Japan. The water-holding capacity (WHC) was determined following the procedures outlined by Kauffman et al. [78]. Additionally, the drip loss was quantified using roughly 2 g of heated material, following the plastic bag technique outlined by Honikel [79]. Subsequently, the cooking loss was calculated using the methodology laid out by Sullivan et al. [80]. The TBARS were quantified using the technique outlined by Witte et al. [81], with the results expressed as milligrams of MDA per kilogram of muscle. The extraction process involved the use of a solution of trichloroacetic acid with a concentration of 20% by weight/volume.

5.10. Immune Response and Metabolizing Cytochrome P450 Enzyme-Related Genes Expression in the Liver

At the end of the experiment, three birds were randomly selected from each treatment, and their liver tissues were immediately removed and frozen at -80°C until RNA extraction. Total RNA was extracted from 50 mg of liver samples homogenized with liquid nitrogen using Trizol and a columnar RNA extraction kit (Invitrogen, PureLink[™] RNA Mini Kit, Thermo Scientific, Wilmington, NC, USA) according to the manufacturer's

protocol. The extracted RNA was quantified using a spectrophotometer (NanoDrop™ 2000, Thermo Scientific, Wilmington, NC, USA) at an absorbance ratio of 260–280 nm. Subsequently, the cDNA was synthesized using a cDNA synthesis kit (iScript™ cDNA Synthesis Kit, BIO-RAD, Hercules, CA, USA) according to the manufacturer's instructions.

The qPCR reaction was carried out using the CFX Connect™ Real-Time PCR System (BIO-RAD, Hercules, CA, USA) with the iTaq Universal SYBR Green supermix 2X (BIO-RAD, Hercules, CA, USA) and specific primers for individual genes (Table 8). Changes in the expression levels of the above genes were measured using the $2^{-\Delta\Delta C_t}$ method and a standard curve, as outlined by Larionov et al. [82].

Table 8. Primer sequences, amplicons, and the related information for quantitative real-time PCR.

Target Gene		Primer Sequences	Product Size (bp)
Housekeeping gene			
GAPDH	Forward	CTGGCATTGCACTGAACGAC	165
	Reverse	CTCCAACAAAGGGTCCTGCT	
Immune-related genes			
IL-6	Forward	GCGGAACCAAGAGCAGAGATGAG	130
	Reverse	CCACGGCAGGACTGGATAATAACC	
IL-8	Forward	GCTGTCCTGGCTCTTCTCCT	120
	Reverse	GCACACCTCTCTGTTGTCTTC	
TNF- α	Forward	CCGTGGTCAGTTTCCATCAGG	117
	Reverse	ACTTTGCAGTTAGGTGACGCT	
P450 (Metabolism of AFB1) genes			
CYP1A1	Forward	AGGACGGAGGCTGACAAGGTG	104
	Reverse	AGGATGGTGGTGAGGAAGAGGAAG	
CYP1A2	Forward	CCACGCAGATCCCAAACGAG	120
	Reverse	TGTGAGGGTACGTCACGAGG	

IL6 = interleukin 6; IL8 = interleukin 8; TNF- α = tumor necrosis factor alpha; CYP1A1 = cytochrome P450 1A1; CYP1A2 = cytochrome P450 1A2.

5.11. Statistical Analysis

The experimental data were analyzed using the analysis of variance (ANOVA) procedure of SAS Enterprise Guide Software V.9.4 (SAS Institute, Cary, NC, USA). The least square means (LSM) were compared using Tukey's test, and a probability level of $p < 0.05$ was considered statistically significant.

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